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IS 548-2 (1976): Methods of sampling and test for oils and fats, Part II: Purity tests [FAD 13: Oils and Oilseeds]



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IS : 548 (Part II) - 1976
(Reaffirmed ~~2008~~)
2010

Indian Standard

METHODS OF SAMPLING
AND TEST FOR OILS AND FATS

PART II PURITY TEST

(Third Revision)

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NEW DELHI 110002

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Indian Standard

METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART II PURITY TESTS

(*Third Revision*)

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AMENDMENT NO. 1 FEBRUARY 1979
TO
IS : 548 (Part II)-1976 METHODS OF SAMPLING
AND TEST FOR OILS AND FATS

PART II PURITY TESTS

(Third Revision)

Alterations

(Page 6, clauses 6.3 and 7.3; page 7, clauses 8.3 and 9.3; page 15, clause 14.3 and page 18, clause 16.5, line 1) — Substitute 'generally sensitive' for 'sensitive'.

(Page 11, clause 11.2.2, first sentence) — Substitute the following for the existing sentence:

' Pour 30 ml of the oil into a 250-ml conical flask and mix well with about 50 ml of water.'

(Page 13, clause 12.2.3, Note):

a) Line 1 — Substitute 'sample' for 'sampling'.

b) Line 3 — Substitute '12.2.3' for '10.2.3'

(Page 13, clause 13.1.2, line 4) — Substitute '27°C is 0.880 7' for '15.5°C is 0.889 8'.

(Page 14, clause 13.2.2, line 2) — Substitute 'litre' for 'titre'.

Addenda

(Page 3, contents, item 18) — Add the following new item after item 18:

' 19. TEST FOR THE PRESENCE OF OIL SOLUBLE COLOURS
IN OILS AND FATS

25'

(Page 6, clause 6.2) — Add the following new note after 6.2:

' NOTE — Test the sample for presence of colouring matter which are chromogenic in presence of hydrochloric acid. For this purpose, take 5 ml of the sample in a 25-ml measuring cylinder provided with a glass stopper and shake with 5 ml of concentrated hydrochloric acid. If there is no development of a pink or red colour in the aqueous layer, apply the test as prescribed in 6.2. If pink or red colour develops in the aqueous layer, remove the red acid layer which collects at the bottom and repeat the procedure until no further colouration takes place. After complete removal of hydrochloric acid layer, perform the test on the sample so obtained as prescribed in 6.2.'

Gr 2

(*Page 10, clause 11.0*) — Add the following new matter at the end of the clause:

‘Method A shall be used as a referee method whereas Method B may be used for routine analysis.’

(*Page 24, clause 18.4*) — Add the following new clauses after 18.4:

‘19. TEST FOR THE PRESENCE OF OIL SOLUBLE COLOURS IN OILS AND FATS

19.0 General — Oil soluble colours are colours (both natural including nature identical and synthetic) soluble in oils and fats.

19.1 Outline of the Method — Oils, fats and other interfering substances are removed from the colours by solvent partition technique using dimethyl formamide and hexane (3 : 1), followed by alumina adsorption. The colours are detected by reversed phase paper chromatography.

19.2 Apparatus

19.2.1 For Extraction — a mechanical shaker.

19.2.2 For Column Chromatography — a chromatographic tube made of glass (2.5 cm in diameter and 30 cm in length).

19.2.3 Separating Funnel — 250 ml capacity.

19.3 Reagents

19.3.1 Hexane

19.3.2 Dimethyl Formamide

19.3.3 Sodium Chloride Solution — saturated.

19.3.4 Alumina Neutral, Brockmann, Activity 1, for Example, Made by National Chemical Laboratory, Pune or of Equivalent Quality — activated at 100°C for 4 hours.

19.3.5 Diethyl Ether

19.3.6 Ethyl Alcohol

19.3.7 Ammonium Hydroxide Solution

19.3.8 Methanol

19.3.9 Petroleum Ether — B. P. 40 to 60°C.

19.3.10 Liquid Paraffin

19.3.11 Whatman No. 1 Filter Paper (Reversed Phase) — Prepared by soaking the paper in 5 percent liquid paraffin in petroleum ether and subsequent drying in air.

19.3.12 Oil Soluble Colours — Butter yellow, Cres orange GN, Sudan IV, Gress Yellow GRN type, etc.

19.3.13 Sodium Hydroxide Solution — 5 percent (*m/v*).

19.3.14 Sulphuric Acid Solution — 13 N.

19.3.15 Stannous Chloride Solution in Hydrochloric Acid — Dissolve 112.8 g of stannous chloride in 170 ml of concentrated hydrochloric acid using heat if necessary. Dilute with water to 1 litre and add a few pieces of tin metal.

19.3.16 Boric Acid Solution — Dissolve 4 g of boric acid in 100 ml of concentrated hydrochloric acid (relative density 1.19).

19.3.17 Anhydrous Sodium Sulphate

19.3.18 Saturated Solution of Antimony Trichloride in Chloroform

19.3.19 Solvent Mixture — Diethyl Ether : Alcohol : water :: 5 : 3 : 2.

19.3.20 Solvent Mixture — 80 percent ethyl alcohol in water.

19.4 Procedure

19.4.1 Extraction — Take about 2 to 3 g of the sample in a conical flask, add approximately 50 ml of hexane and then shake for about 10 minutes in a mechanical shaker. Filter it and concentrate the filtrate to about 10 ml.

19.4.1.1 Qualitative test for presence of synthetic colours — Divide a portion of the hexane layer into 4 parts and treat with either 13 N sulphuric acid or concentrated hydrochloric acid and water mixtures 4 : 1, 2 : 1 or 1 : 1 respectively. If the acid layer or the whole extract changes shade or more particularly develops pink to reddish violet colour, the synthetic oil soluble colour may be suspected.

19.4.2 Separation of Colours from Interfering Materials — Transfer the concentrated extract to a separating funnel and add to it dimethyl formamide (DMF) about three times the volume of the concentrated extract and shake vigorously. Keep it for a while for separation of the layers. The DMF layer contains the natural colour as well as the synthetic colours (if present), leaving oils and fats in the hexane layer. Now take the DMF layer and again shake it with hexane after addition of about 20 ml of water. Retransfer colours (both natural and synthetic) this time to the hexane layer. If emulsion is formed add saturated sodium chloride solution. Concentrate the hexane layer and preferably repeat the above mentioned procedure. If oils and fats still linger in the sample, these will float above the coloured extract when the hexane layer is concentrated to 0.5 ml. If oil is present repeat the above procedure till free from oil. Make the hexane solution to 10 ml. If curcumin or turmeric is suspected, shake the residual DMF layer after extraction with and separation of hexane with diethyl ether and an extra amount of water, if necessary and collect the ether layer. Add the ether layer to the previous hexane. By

this treatment any minute curcumin and turmeric will come to the ether layer.

19.4.3 Separation of Natural Colours from Synthetic Oil Soluble Colours — Clamp the chromatographic tube and pour 40 ml of hexane with the non-greasy stopcock in closed position. Place a cotton plug (if no fritted glass is fixed in the tube) firmly at the bottom of the tube. Pour 40 ml of hexane followed by anhydrous sodium sulphate to form a 2 to 3 cm layer. Now open the stopcock to give a trickle of solvent and tap the tube. Pour alumina sufficient to give a column of 10 cm length into the solvent with constant tapping. This will give a channel-free column. It is important that the top of the alumina is always under the solvent. When the solvent layer reaches about 1 cm above the alumina, pour the hexane extract of colours carefully into the tube. Collect the eluent in a beaker. Continue the elution with four batches of 10 ml hexane. Concentrate the eluate containing oil soluble synthetic colours. After elution with hexane pass 40 ml of another eluting solvent, namely, petroleum ether : acetone (1 : 1) for complete elution.

19.4.4 Detection of Synthetic Oil Soluble Colours — Cut a reversed phase chromatography paper to a size of 20 × 20 cm. Then draw a straight line by a lead pencil on the paper leaving away 3 cm from the bottom. Spot the concentrated extract (say 10 μ l or more according to the colour present on the solution) and also some known oil soluble colours on the line drawn on the reversed phase chromatography paper leaving 1 cm distance from each other. Then make the paper to a cylindrical shape, staple it and dip in any one of the solvents (see 19.3.19 and 19.3.20) for ascending chromatography. Run for 10 cm. The development in the solvent given under 19.3.19 takes 45 minutes and that in solvent prescribed under 19.3.20 takes one hour. Take out the paper, dry in air and detect the colour by comparing with the known colour in respect of shade and Rf values.

19.4.5 Detection of Natural Colours — Elute the natural colours absorbed on the column by the solvent methanol : ammonium hydroxide (9 : 1) and then examine for natural colours. If colour of the column changes to red-violet after passing the above mentioned solvent, it confirms the presence of curcumin or turmeric.

19.4.5.1 Detection of annatto, turmeric and curcumin — Evaporate the extracted layer (see 19.4.5) to dryness, add 10 ml petroleum ether and shake a 7 ml portion of the solution with sodium hydroxide solution. Take out the alkali layer and divide it into two portions to check for the presence of annatto, turmeric and curcumin. Dilute the first portion with equal volume of water, add a piece of clean white adsorbent cotton to it and keep it overnight. If after washing gently in water the cotton piece continues to retain straw colour which turns pink by a drop of stannous chloride solution, it confirms the presence of annatto. To the second

portion add boric acid solution to make the solution acidic. If red colour appears, turmeric is present and if pink colour appears it indicates the presence of curcumin. Sometimes turmeric and curcumin are not eluted if present in small amount. Notice the change of colour of the column for their detection.

19.4.5.2 Detection of carotène — To the non-alkali treated portion add saturated solution of antimony trichloride in chloroform. If blue colour appears, carotene is present.'

(CAFDC 5)

AMENDMENT NO. 2 MAY 1984

TO

IS:548(Part 2)-1976 METHODS OF SAMPLING AND TEST
FOR OILS AND FATS

PART 2 PURITY TESTS

(Third Revision)

Alterations

(Page 8, clause 10.2.7) - Substitute '125 watts with a maximum emission at 350 mm' for '125 watts.'

(Page 11, clause 12.0) - Substitute the following for the existing clause:

'12.0 *General* - Two methods are prescribed. Method A may be used for rapid detection of mineral oil in vegetable oils and fats. It is sensitive when mineral oil is present to the extent of 1 percent or more. Method B shall be used where confirmation is desirable; it is also sensitive to 1 percent of mineral oil in other oils. Oils with high contents of unsaponifiable matter may give a false positive response in both the methods.'

(Page 18, clause 17.2.5) - Substitute the following for the existing clause:

'17.2.5 *Alcoholic Orthophosphoric Acid Solution* - 50 percent (v/v) solution of orthophosphoric acid (85-88 percent) in 95 percent ethanol.'

(Page 18, clause 17.3.1) - Substitute the following for the existing clause:

'17.3.1 *TLC Applicator*'

(Page 18, clause 17.3.4) - Substitute the following for the existing clause:

'17.3.4 *TLC Pipettes or Capillaries* - approximately 50 ml capacity.'

(Page 18, clause 17.4.1, line 3) - Substitute '250 μm ' for '250 nm'.

Addenda

(Page 14, clause 13.2.3, Note) - Redesignate the existing note as Note 1 and add the following note after it:

'Note 2 - The test may not be reliable in presence of other oils containing high content of component palmitic, stearic and very long chain fatty acids, hydrocarbons and added mineral oils.'

(Page 18, clause 17.3.4) - Add the following new clause after '17.3.4':

'17.3.5 *Glass Plates* - 20 x 20 cm.'

(CAFDC 5)

AMENDMENT NO. 3 SEPTEMBER 1984

TO

IS:548(Part 2)-1976 METHODS OF SAMPLING AND
TEST FOR OILS AND FATS

PART 2 PURITY TESTS

(Third Revision)

Addendum

(Page 6, clause 7.0) - Add the following at the
end of this clause:

'In view of the fact that even cottonseed oil after
some treatment (heating) may not respond to this
test, it is desirable that this test be used as a
screening test only. In taking a view on a particular
edible oil, all the analytical characteristics should
be considered for the presence of cottonseed oil'.

(CAFD 5)

AMENDMENT NO. 4 OCTOBER 1985

TO

IS:548(Part II)-1976 METHODS OF SAMPLING AND TEST
FOR OILS AND FATS

PART II PURITY TESTS

(Third Revision)

(Page 24, clause 16.4) - Add the following new clause after 18.4:

'18.4.1 The presence of animal fat may be suspected if the melting point of sterol acetate is observed as 115°C or less.'

NOTE - The presence of animal fat in vegetable oils and fats shall be confirmed by TLC/GLC method *(under consideration)*.'

AMENDMENT NO. 5 MAY 1991
TO
IS 548 (Part 2) : 1976 METHODS OF SAMPLING
AND TEST FOR OILS AND FATS
PART 2 PURITY TESTS

(Third Revision)

(Page 9, clause 10.4.1) — Substitute the following for the existing clause:

'10.4.1 Extraction — Take 2 ml of oil sample into a 10 ml volumetric flask. Add 1 ml of 6 N hydrochloric acid (AR Grade) and 1 ml ethanol (AR Grade) and shake vigorously for two minutes on an electric mixer. Hold the flask in a hot water bath maintained at 60-70°C till the aqueous layer is clear. Filter through moistened filter paper (Whatman No. 1). Re-extract the oil left on the filter paper with 2 ml of hydrochloric acid and ethanol mixture (1 : 1 v/v). Collect the combined filtrates in a 10 ml beaker and evaporate it to dryness on a hot water bath.'

AMENDMENT NO. 6 APRIL 1995
TO
IS 548 (Part 2) : 1976 METHODS OF SAMPLING AND
TEST FOR OILS AND FATS
PART 2 PURITY TESTS

(Third Revision)

[*Page 7, clauses 9.1 to 9.3*] — Substitute the following for the existing:

“9.1 The test method prescribed in IS 548 (Part 2/Sec 9) : 1988 ‘Methods of sampling and test for oils and fats : Part 2 Purity tests, Section 9 Test for presence of *Karanja (Pungam)* oils in other oils (*fourth revision*)’, shall be followed.”

(FAD 44)

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AMENDMENT NO. 7 SEPTEMBER 1996
TO
IS 548 (Part 2) :1976 METHODS OF SAMPLING
AND TEST FOR OILS AND FATS

PART 2 PURITY TESTS

(Third Revision)

(Page 15, clause 15) — Substitute the following for the existing clause and its sub-clauses:

‘15 DETECTION OF CASTOR OIL IN EDIBLE OILS

15.0 Principle — Triricinolein a characteristic and predominate triglyceride component of castor oil is separated on silica gel TLC and visualised by iodine vapours.

15.1 Apparatus

15.1.1 Slides — Microscopic slides (7.6 cm × 1.5 cm) or glass plates of 20 cm × 5 cm or 20 cm × 10 cm.

15.1.2 Developing Tank — A tall beaker of atleast 10 cm height/TLC developing chamber.

15.1.3 Visualisation Tank — A dry tank saturated with iodine vapour by placing a few crystals at the bottom and leaving for one hour.

15.2 Reagents/Chemicals

15.2.1 Silica Gel — Silica gel-G.

15.2.2 Developing Solvent — Petroleum ether (BP 40 to 60°C), Diethyl ether and acetic acid (60 : 40 : 2, v/v).

15.3 Procedure

15.3.1 At one end of the silica gel-G coated slides (*see 12*) apply 5 µl of the oil sample as a one percent solution in chloroform and place the plate in a breaker/chamber containing the developing solvent and then cover with a watch glass or a glass plate for a chamber and allow the solvent to travel up to a height of 5 to 7 cm (about 6 min) or 10 cm for bigger plate. Remove the plate from the tank, dry in air and place in the visualisation tank containing iodine vapour.

Amend No. 7 to IS 548 (Part 2) : 1976

Occurrence of a spot with an Rf value of about 0.25 shows the presence of castor oil. All the other spots will be above this spot. A prepared sample of an oil containing one percent added castor oil may be run along side for convenience in identifying the spot. The spot shall be noted in the visualisation tank, since it fades on removing. This method has a sensitivity of one percent.

NOTE — This method is specific for castor oil, but rancid or oxidised groundnut oil gives a streak. Hence care should be taken when applying the TLC test to rancid oils and interpretation of result. In such cases the rancid oil has to be purified by 'refining' as given in 15.3.2.

15.3.2 TLC method for detection for castor oil and its differentiation from rancid oils: The suspected rancid oil (5 ml) may be taken in a round bottom flask and add activated charcoal (2 g). The content is mixed thoroughly and heated on boiling water bath for about 30 min with constant shaking. The bleached oil is filtered on a filter paper to separate the charcoal. The filtered oil may now be passed through a mini column packed with neutral alumina (10 g) using hexane (50 ml) as eluent. This bleached and neutralised oil may be spotted on the TLC plate following the earlier TLC procedure given for the detection of castor oil spotting castor oil besides as reference standard.'

(FAD 44)

Indian Standard
**METHODS OF SAMPLING
AND TEST FOR OILS AND FATS**
PART II PURITY TESTS
(Third Revision)

0. F O R E W O R D

0.1 This Indian Standard (Part II) (Third Revision) was adopted by the Indian Standards Institution on 25 November 1976, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Chemical Division Council and the Agricultural and Food Products Division Council.

0.2 This standard was first published in 1954 and subsequently revised in 1964 and 1974. It prescribes qualitative tests for determining the purity of oils and fats. In the third revision, sensitivity limits of modified Baudouin test, Halphen test, hexabromide test, tests for presence of mineral oils, castor oil, *KUSUM* oil and other oils containing cyanogenic compounds have been included on the basis of collaborative inter-laboratory investigations. A paper chromatographic method for detection of argemone oil has been prescribed in place of conventional ferric chloride test. In addition, tests for the presence of *NEEM* oil and other oils in castor oil have also been included. The sensitivity limits of various tests specified in this standard are based on use of raw oils and may decrease on processing of the oils, for example, refining, bleaching, deodourization and other treatments.

0.3 This standard is intended to introduce in India uniform methods of sampling and test for oils and fats. It does not deal with the specifications of the materials, but prescribes only the methods of determining whether the material conforms to the requirements of the individual standard and thus forms a necessary adjunct to the series of Indian Standard specifications for individual oils and fats.

0.4 In the preparation of the third revision of this standard, substantial assistance has been derived from data supplied by Regional Research Laboratories, Hyderabad and Bhubaneswar, Oil Technological Research Institute, Anantapur, Central Food Laboratory, Calcutta, M/s Hindustan Lever Ltd, Bombay and Directorate of Marketing and Inspection, Nagpur. The assistance so derived is gratefully acknowledged.

0.5 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard (Part II) prescribes methods of sampling and purity tests for the oils and fats.

1.1.1 The purity of oils and fats is determined indirectly by testing the absence of other oils or adulterants.

2. TERMINOLOGY

2.1 For the purpose of this standard, the definitions given under 2, of IS : 548 (Part I)-1964† shall apply.

3. SAMPLING

3.1 Representative samples of the material shall be drawn as prescribed in 3 of IS : 548 (Part I)-1964‡.

4. QUALITY OF REAGENTS

4.1 Unless otherwise specified, pure chemicals and distilled water (see IS : 1070-1960‡) shall be employed in tests.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the results of analysis.

5. GENERAL DIRECTIONS

5.1 Melt the sample if it is not already liquid, mix it thoroughly and filter through a filter paper to remove any impurities and the last traces of moisture.

6. TEST FOR THE PRESENCE OF SESAME OIL (MODIFIED BAUDOUIN TEST)

6.0 General — The development of a permanent pink colour with furfural solution in the presence of hydrochloric acid indicates the presence of sesame oil.

6.1 Reagents

6.1.1 Hydrochloric Acid — fuming, relative density 1.19.

*Rules for rounding off numerical values (revised).

†Methods of sampling and test for oils and fats: Part I Sampling, physical and chemical tests (revised).

‡Specification for water, distilled quality (revised).

6.1.2 Furfural Solution — 2 percent solution of furfural, distilled not earlier than 24 hours prior to the test, in rectified spirit (*see* IS: 323-1959*). The reagent is stable up to 3 months if kept in a refrigerator.

6.2 Procedure — Take 5 ml of the oil or melted fat in a 25-ml measuring cylinder (or test tube) provided with a glass stopper, and add 5 ml of hydrochloric acid and 0.4 ml of furfural solution. Insert the glass stopper and shake vigorously for two minutes. Allow the mixture to separate. The development of a pink or red colour in the acid layer indicates presence of sesame oil. Confirm by adding 5 ml of water and shaking again. If the colour in acid layer persists, sesame oil is present. If the colour disappears it is absent.

6.3 Sensitivity — This test is sensitive to the extent of 0.2 percent of sesame oil in other oils.

7. TEST FOR THE PRESENCE OF COTTONSEED OIL (HALPHEN TEST)

7.0 General — The development of a red colour on heating the oil with a solution of sulphur in carbon disulphide indicates the presence of cottonseed oil. The test is also given by hempseed oil, kapok oil, and oils and fats containing fatty acids of cyclopropenoid structure.

7.1 Reagents

7.1.1 Sulphur Solution — Prepare a one percent (*m/v*) solution of sulphur in carbon disulphide and then add an equal volume of amyl alcohol.

7.2 Procedure — Take about 5 ml of the oil or melted fat in a test tube and add to it an equal volume of the sulphur solution. Mix thoroughly by shaking and heat gently in a water-bath (70 to 80°C) for a few minutes with occasional shaking until the carbon disulphide has boiled off and the sample stops foaming. Place the tube in an oil-bath or a saturated brine-bath maintained at 110 to 115°C, and hold for 1 to 2 hours. A red colour at the end of this period indicates the presence of cottonseed oil.

7.3 Sensitivity — This test is sensitive to the extent of 0.5 percent of cottonseed oil in other oils.

8. TEST FOR THE PRESENCE OF LINSEED OIL (HEXABROMIDE TEST)

8.0 General — The formation of a precipitate of hexabromide when the oil is treated with bromine in chloroform and then with alcohol and ether indicates the presence of linseed oil. The test is also given by fish oils, and oils and fats containing highly unsaturated fatty acids. Sometimes soyabean oil and low erucic rapeseed oil depending upon linolenic acid content may also give positive response to this test.

*Specification for rectified spirit (*revised*).

8.1 Reagents

8.1.1 *Chloroform*

8.1.2 *Liquid Bromine*

8.1.3 *Rectified Spirit* — See IS : 323-1959*.

8.1.4 *Ether* — See solvent grade of IS : 336-1964†.

8.2 Procedure — Pipette 1 ml of the oil in a dry test tube which may be fitted with a ground glass stopper. Add 5 ml of chloroform. Add about 1 ml of bromine dropwise till the mixture is deep red in colour and cool the test tube in an ice water-bath. Add about 1.5 ml of rectified spirit dropwise while shaking the mixture until the precipitate which is first formed just dissolves and then add 10 ml of ether. Mix and place the tube in the ice water-bath for 30 minutes. Appearance of a precipitate indicates the presence of linseed oil.

NOTE 1 — This test is not applicable for detecting linseed oil in *MAHUA* oil.

NOTE 2 — The use of Lunge-Rey pipette is suggested for handling and addition of bromine.

NOTE 3 — Cool the tube containing oil-chloroform mixture in an ice water-bath and while adding bromine maintain it cool.

8.3 Sensitivity — This test is sensitive to the extent of 1.0 percent of linseed oil in other oils.

9. TEST FOR THE PRESENCE OF *KARANJA* (*PUNGAM*) OIL AND OTHER OILS CONTAINING PHENOLIC SUBSTANCES

9.0 General — The non-saponifiable components of these oils react with antimony trichloride to form yellow to orange coloured complexes.

9.1 Reagent

9.1.1 *Antimony Trichloride Solution* — 20 percent (*m/v*) in chloroform. The reagent is prepared by weighing antimony trichloride crystals, adding to chloroform and shaking for a few minutes till the crystals dissolve.

9.2 Procedure — Take one drop of the oil sample in a small test tube or in a depression of porcelain tile. Add 1 to 2 ml of antimony trichloride reagent. An immediate characteristic canary yellow to orange yellow colour shows the presence of *KARANJA* (*PUNGAM*) oil in the sample.

9.3 Sensitivity — This test is sensitive to the extent of 3 percent of *KARANJA* (*PUNGAM*) oil in other oils.

*Specification for rectified spirit (*revised*).

†Specification for ether (*revised*).

10. TEST FOR THE PRESENCE OF ARGEMONE OIL BY PAPER CHROMATOGRAPHIC METHOD

10.0 General

10.0.1 Argemone (*Argemone mexicana* Linn) oil is sometimes present as a contaminant in edible oils, particularly in mustard oil. The oil is reported to cause toxic symptoms and the toxicity is ascribed to the presence of an alkaloid sanguinarine in the oil. Besides sanguinarine, argemone oil also contains dihydro-sanguinarine.

10.0.2 The paper chromatographic method is sensitive and provides an unambiguous method of detecting and confirming the presence of sanguinarine. Realizing the need for a quick and sensitive method of analysis the different steps of the chromatographic procedure have been standardized to reduce the time required for the test.

10.1 Outline of the Method — The procedure consists of extraction of alkaloids and development of chromatogram. The alkaloid extract is concentrated by evaporation before paper chromatography. The paper is viewed under ultraviolet light and spots of sample under test are observed.

10.2 Apparatus

10.2.1 Graduated Tapered Centrifuge Tubes — 10 ml capacity.

10.2.2 Filter Paper — Whatman chromatographic grade No. 4 or equivalent.

10.2.3 Capillary Pipettes — The capillary has internal diameter of 1 mm. It is convenient to draw the capillary from a glass tubing of 0.3 to 0.4 cm internal diameter.

10.2.4 Chromatography Tank or Air-Tight Glass Chamber with Glass Lid — Chromatography tank of 36 cm height, 28 cm breadth and 28 cm length or any other suitable dimension may be used.

10.2.5 Glass Boat — of suitable size for holding the developing solvent.

10.2.6 Flask — with side arm connection of 250 ml capacity.

10.2.7 Ultraviolet (U.V.) Lamp — 125 watts.

10.2.8 Hot Air Blower

10.3 Reagents

10.3.1 Diethyl Ether

10.3.2 Concentrated Hydrochloric Acid — relative density 1.19.

10.3.3 Concentrated Sulphuric Acid — 98 percent (v/v),

10.3.4 n-Butanol — laboratory reagent grade.

10.3.5 Sodium Hydroxide Solution — 1 N.**10.3.6 Sulphuric Acid — 1 N.****10.3.7 Sodium Chloride**

10.3.8 Ethereal Hydrochloric Acid (1 N) — Place 30 to 40 g of sodium chloride in the flask with side arm. Add sufficient concentrated hydrochloric acid to just cover the salt. Close the flask with a rubber stopper to which a separatory funnel is fixed. Add sulphuric acid drop by drop through the funnel. Pass hydrochloric acid gas through a scrubber containing concentrated sulphuric acid in ice-cooled ether. Pass the gas for about 45 minutes or so until the normality of the acidified ether is above 1 N. (Remove 5 ml of acidified ether, add 10 ml of sodium hydroxide solution, warm to remove ether and back titrate the excess with 1 N sulphuric acid.) The reagent should be stored in a refrigerator.

10.3.9 Mobile Phase — Mix 100 ml of *n*-butanol, 20 ml of concentrated hydrochloric acid and 35 ml of water in a separating funnel. Allow to settle and use the top clear solution.

10.4 Procedure

10.4.1 Extraction — Take 1 ml of the filtered oil in 10 ml graduated tapered centrifuge tube. Add 2 ml of ethereal hydrochloric acid reagent and shake to dissolve the oil. To this solution add one drop of concentrated hydrochloric acid and shake acid extraction in the tube rather vigorously by jerking action of the wrist, till the acid drop is dispersed completely. Allow the tube to stand for a short while when the initial turbid solution becomes clear. The ether layer can now be decanted. Wash the tube two or three times with fresh ether (2 ml each time) to remove the oil. The aqueous layer tends to cling to the sides of the tube and ultimately it is retained in the form of a drop at the bottom of the tube. Remove the last traces of ether by warming the tube in the water-bath.

10.4.2 Spotting — Cut the chromatographic filter paper according to the requirement of the chromatographic chamber with length along the machine direction. Spot a drop of the acid layer on the paper by means of capillary tube. Dry the spot after each application using hot air blower to avoid spreading. Transfer the entire quantity of the acid layer to the paper by repeated spotting and drying. Spot each sample above 4 cm from the end of the paper. The distance between two sample spots should be about 2 cm. View the paper under U.V. light in a dark room. If the spot is non-fluorescent, argemone oil if present, is below the sensitivity limit, namely, less than 50 ppm. Roughly 15 minutes would be sufficient to complete the sequence of operations, namely, extraction, spotting, drying and viewing under U.V. light. The analyst can conveniently handle three test samples during the period.

10.4.3 Paper Chromatography — When the spots are fluorescent under U.V. light, the paper shall be developed to confirm the presence or absence of sanguinarine. It has been observed that cottonseed, sesame and solvent extracted oils also give a fluorescent spot when viewed under U.V. light. The colour of fluorescence is, however, pale blue or green and quite different from the bright orange fluorescence of standard sample containing argemone oil. Irrespective of the colour of fluorescence, the paper has to be developed for further confirmation. The paper is developed by standard procedure employing the solvent system butanol : concentrated hydrochloric acid : water :: 100 : 20 : 38 in the descending manner. The mobile phase is allowed to travel to a distance of about 10 cm. After development the paper is dried in air. Nylon thread and plastics clips are convenient to hold the paper during drying. The paper is viewed under U.V. light. A bright orange spot at Rf 0.56 indicates the presence of argemone oil.

NOTE 1 — Sometimes instead of orange spot, a violet spot is also seen. The presence of violet spot at the same Rf also indicates the presence of argemone oil.

NOTE 2 — If desired a standard stock solution containing 50 ppm of pure argemone oil (expressed from genuine *Argemone mexicana* Linn. seeds) in refined groundnut oil may be prepared and tested as prescribed in 10.4 and spots obtained may be compared with those obtained by using sample under test.

10.4.4 The total time for analysis including the paper development part is roughly 2.5 hours.

10.5 Sensitivity — This test is sensitive to the extent of 50 ppm of expressed argemone oil in other oils.

11. TEST FOR THE PRESENCE OF HYDROCYANIC ACID

11.0 General — Hydrocyanic acid is sometimes present as an impurity in synthetic allyl isothiocyanate which is commonly used as an adulterant to enhance the flavour of poor quality mustard oil. Two methods have been prescribed for the purpose of this test.

11.1 Method A — The hydrocyanic acid in the oil heated over water-bath is displaced by bubbling air and is absorbed in potassium hydroxide solution. The cyanide is then tested with ferric chloride solution.

11.1.1 Reagents

11.1.1.1 Potassium hydroxide solution — approximately 2 N.

11.1.1.2 Lead acetate solution — approximately 2 N.

11.1.1.3 Ferrous sulphate solution — approximately 2 percent.

11.1.1.4 Hydrochloric acid — see IS : 265-1962*.

*Specification for hydrochloric acid (revised).

11.1.1.5 Ferric chloride solution — 20 percent (m/v) in water to which sufficient hydrochloric acid has been added to prevent hydrolysis.

11.1.2 Procedure — Heat about 50 ml of the oil in a distillation flask by placing it on a water-bath. During heating, pass through the oil, for about 30 minutes, air which has been purified by scrubbing through solutions of potassium hydroxide and lead acetate. Connect the distillation flask to an absorption tube containing 5 ml of potassium hydroxide solution. The air bubbling through the oil carries with it the hydrocyanic acid and this is absorbed by the potassium hydroxide solution. Shake the solution with a few drops of ferrous sulphate solution, acidify with a few drops of hydrochloric acid, and warm gently for 5 minutes. Filter and add a few drops of ferric chloride solution. A blue or bluish green colour or precipitate in the solution indicates the presence of cyanides.

11.2 Method B — This method is based on the reaction of hydrocyanic acid on picric acid paper, which acquires a red colour in the presence of that acid.

11.2.1 Reagents

11.2.1.1 Picric acid paper — Soak a filter paper (Whatman No. 1 or equivalent) in a saturated aqueous solution of picric acid, draining the excess liquid and drying the dyed paper in air. The paper shall be prepared freshly before use.

11.2.1.2 Tartaric acid solution — 10 percent (m/v).

11.2.1.3 Sodium carbonate solution — 5 percent (m/v).

11.2.2 Procedure — Pour 15 to 30 ml of the oil depending on its hydrocyanic acid content into a 250-ml conical flask and mix well with about 50 ml of water. Add 15 ml of tartaric acid solution and mix. Stopper the flask with a velvet cork from which hangs a picric acid paper (about 75 mm long) previously wetted with a drop of sodium carbonate solution. Place the flask on a hot water-bath by the side of the steam vent and not directly on the steam for 30 to 45 minutes. In the presence of hydrocyanic acid, the picric acid paper acquires red colour. Ignore pink or light reddish hue which may, at times, appear at the periphery of the picric acid paper.

12. TEST FOR THE PRESENCE OF MINERAL OIL

12.0 General — Two methods are prescribed. Method A may be used for rapid detection of mineral oil in vegetable oils and fats. It is sensitive when mineral oils is present to the extent of 1 percent. Oils with very high contents of unsaponifiable matter may fail the test. Method B shall be used where confirmation is desirable; it is also sensitive to 1 percent of mineral oil in other oils.

12.1 Method A (Holde Test) — The presence of mineral oil is indicated by the development of turbidity when distilled water is added to a freshly made alcoholic solution of the soap formed by the oil.

12.1.1 Reagent

12.1.1.1 Alcoholic caustic potash solution — approximately 0.5 N.

12.1.2 Procedure — Take 25 ml of the alcoholic caustic potash solution in a conical flask and add 1 ml of the sample of oil. Boil on a water-bath using an air or water cooled condenser till the solution becomes clear and no oily drops are found on the sides of the flask. Take out the flask from the water-bath, transfer the contents to a wide-mouth warm test tube and carefully add 25 ml of boiling distilled water along the sides of the test tube. Keep on shaking the tube lightly from side to side during the addition. The presence of mineral oil is indicated by turbidity in the solution, the depth of turbidity depending on the percentage of mineral oil present.

12.2 Method B — Mineral oils are detected because, being non-polar, they move faster on thin-layer chromatographic plates than triglycerides.

12.2.1 Apparatus

12.2.1.1 Slides — two clean microscope slides (7.6 × 2.5 cm). Glass plates of 20 × 5 cm or 20 × 10 cm may also be used instead of microscope slides.

12.2.1.2 Developing tank — a tall-form beaker of at least 10 cm height.

12.2.1.3 Ultraviolet lamp (3650 Å) — This shall be placed in a darkened enclosure.

12.2.2 Reagents

12.2.2.1 Silica gel containing binder — passing 75 micron IS Sieve; this is a mixture of silica gel (85 g) and calcium sulphate (15 g) as binder; the mixed material is available commercially.

12.2.2.2 Petroleum ether — of boiling range 40 to 60°C or 60 to 80°C.

12.2.2.3 Indicator — a 0.2-percent solution of 2',7'-dichlorofluorescein in 95 percent ethanol.

12.2.3 Procedure — Hold the two slides together face to face and dip them in a slurry of silica gel (45 g) in a mixture of chloroform and methanol (80 ml + 20 ml). Withdraw the slides, separate them and allow to dry in air for 10 minutes. At one end of the coated slide apply 10 µl (1 000 µg) of the oil sample as a 10-percent solution in chloroform using a capillary (melting point) tube. Dry briefly, and place the slide in the developing tank containing 3 ml of petroleum ether. Cover with a watch glass and allow the solvent to travel for 6 cm from the origin (about 4 minutes).

Remove from the tank, dry in air, spray with the indicator solution and view under ultraviolet light. Occurrence of a yellow fluorescent spot on the solvent front indicates the presence of mineral oil. The vegetable oil forms a yellow streak about 2 to 3 cm long from the point of spotting (see Note).

NOTE — If desired a standard sampling containing 1 percent by mass of white oil (see IS : 1083-1957*) in a sample of pure oil under test may be prepared and tested simultaneously as reference sample as prescribed in 10.2.3.

13. TEST FOR THE PRESENCE OF GROUNDNUT OIL [BELLIER TURBIDITY TEMPERATURE TEST (ACETIC ACID METHOD)]

13.0 General — Oils containing arachidic acid give a precipitate at a particular temperature which is specific for that oil, when their alcoholic soap solution is treated with dilute acetic acid or hydrochloric acid.

13.1 Reagents

13.1.1 Purified Rectified Spirit — Reflux 1.2 litres of rectified spirit (conforming to IS : 323-1959†) for 30 minutes in a distilling flask with 10 g of caustic potash and 6 g of granulated aluminium (or aluminium foil). Distil and collect one litre after discarding the first 50 ml. Use this purified rectified spirit for preparation of all the reagents.

13.1.2 Alcohol — 70 percent (*v/v*). Dilute the purified spirit with the requisite quantity of water and adjust the strength to 70 percent (*v/v*) by relative density determination and reference to the alcohol table. The relative density of 70 percent alcohol at 15.5°C is 0.8898. The final strength should be checked accurately.

13.1.3 Alcoholic Potash Solution — 1.5 N. Dissolve 8.5 g of caustic potash in the purified rectified spirit and dilute to 100 ml with this spirit. It is preferable to keep this solution in a dark bottle.

13.1.4 Dilute Acetic Acid — Mix one volume of glacial acetic acid with 2 volumes of water.

13.1.5 Phenolphthalein Indicator — Dissolve 0.5 g of phenolphthalein in 50 ml of purified rectified spirit and mix the solution with 50 ml of water.

13.2 Procedure

13.2.1 Measure with the aid of a pipette 1 ml of the filtered sample of oil in a flat-bottom 100-ml flask (preferably with a long neck), add 5 ml of alcoholic potash solution and *saponify completely* by heating over a boiling

*Specification for white oil, light, technical.

†Specification for rectified spirit (revised).

water-bath using an air condenser (about 1.3 m long) to avoid loss of alcohol as far as possible. Complete saponification usually takes about 10 minutes. During saponification swirl the flask several times. Cool, add 0.1 ml of phenolphthalein indicator, neutralize exactly by adding *carefully* dilute acetic acid and then add an extra amount of 0.4 ml (accurately measured). Add 50 ml of 70 percent alcohol and mix.

13.2.2 Transfer a portion of the solution to a 10 × 2.5-cm diameter test tube, which is supported in a titre bottle to serve as an air jacket. This bottle can be cooled by immersing in a cold water-bath. Fit a thermometer (0 to 60°C, reading to 0.2°C, accurately calibrated) into the test tube, with the aid of a velvet cork, in such a way that the bulb of the thermometer is immersed in the liquid but does not touch the bottom of the test tube. Heat the test tube gently over the water-bath until the temperature reaches 50°C and the solution is clear. Allow the test tube to cool in air with frequent shaking until the temperature falls gradually to 40°C (in case of pure groundnut oil turbidity appears at 39 to 41°C). Then cool the test tube with constant shaking by occasional immersion in a cooling bath maintained at $15 \pm 1^\circ\text{C}$ so that the temperature drops roughly at the rate of 2°C per minute. Note the temperature at which the first distinct turbidity appears which is the turbidity temperature. This turbidity temperature is confirmed by a little further cooling which would result in deposition of the precipitate.

13.2.3 Dissolve the precipitate by gently heating the contents to 50°C over the water-bath, again cool as described in 13.2.2 and make a duplicate determination of the turbidity temperature. The mean of the two values is taken as the true turbidity temperature.

NOTE — It is essential that stirring be continuous and moderate while the contents are being cooled in the cooling bath. Violent shaking or agitation should be avoided as it will affect the result adversely.

14. TEST FOR THE PRESENCE OF KUSUM OIL AND OTHER OILS CONTAINING CYANOGENIC COMPOUNDS

14.0 General — Such oils produce a green to blue colour when a solution of the soap prepared from them is treated with ferric chloride, ferrous sulphate and hydrochloric acid.

14.1 Reagents

14.1.1 Sodium Hydroxide Solution — 50 percent (*m/v*).

14.1.2 Ferric Chloride Solution — 10 percent (*m/v*).

14.1.3 Ferrous Sulphate Solution — 20 percent (*m/v*).

14.1.4 Hydrochloric Acid — relative density 1.19 (see IS : 265-1962*).

14.1.5 Ethyl Alcohol — 95 percent (by volume), or rectified spirit (see IS : 323-1959†).

14.1.6 Common Salt — clean crystals (see IS : 253-1970‡), powdered.

14.2 Procedure

14.2.1 Take about 5 ml of the oil or melted fat in a 100-ml beaker and add to it 8 ml of ethyl alcohol and 1.3 ml of sodium hydroxide solution. Saponify the oil by placing the beaker in a boiling water-bath and stirring the contents with a glass rod. Care should be taken to ensure that saponification is complete, at which stage the solution is clear and bright. Evaporate off the alcohol as completely as possible by briskly stirring under a gentle stream of clean dry air. Dissolve the soap in 20 ml of water, add about 3 g of salt and stir until the soap grains out. Cool to about 20°C, add 1 ml of ferric chloride solution and 0.5 ml of ferrous sulphate solution, and mix well. Add slowly about 4 ml of hydrochloric acid, mixing thoroughly and allow to stand for 10 minutes. The development of a green, deep green, greenish-blue or deep blue colour in the aqueous layer indicates the presence of *KUSUM* oil or any other oil containing cyanogenic compounds.

14.2.2 If the test prescribed in 14.2.1 is positive, carry out the test for hydrocyanic acid (11) also.

14.3 Sensitivity — This test is sensitive to the extent of 0.5 percent of *KUSUM* oil in other oils.

15. TEST FOR THE PRESENCE OF CASTOR OIL

15.0 General — The turbidity test described in Method A may be used for the rapid detection of castor oil in other oils. Method A is sensitive to the extent of 1 percent of castor oil, but badly oxidized vegetable oils, *MAHUA* oil and some varieties of sesame oil may also respond. A positive response shall be confirmed by Method B based on thin layer chromatography. This is specific for castor oil, and has a sensitivity of 1 percent.

15.1 Method A — When castor oil is present in other oils, treatment of a petroleum ether solution of the oil with sulphuric acid, in the presence of ammonium molybdate, causes a turbidity to appear.

*Specification for hydrochloric acid (revised).

†Specification for rectified spirit (revised).

‡Specification for edible common salt (second revision).

15.1.1 Reagents

15.1.1.1 Acidified petroleum ether — Add 2 ml of concentrated hydrochloric acid to 100 ml of petroleum ether (boiling range 40 to 60°C or 60 to 80°C).

15.1.1.2 Sulphuric acid-molybdate reagent — In 100 ml of concentrated sulphuric acid dissolve 1.25 g of ammonium molybdate.

15.1.2 Procedure — In a clean, dry test tube take about 1 ml of the oil or melted fat and add 10 ml of acidified petroleum ether. Shake vigorously for 2 minutes and add one drop of the molybdate reagent. Instantaneous development of white turbidity indicates the likely presence of castor oil.

15.2 Method B — A triglyceride constituent of castor oil, perhaps triricinolein, gives a spot with a characteristic R_f value by suitable thin layer chromatography.

15.2.1 Apparatus

15.2.1.1 Slides — two clean microscope slides (7.6 × 2.5 cm). Glass plates of 20 × 5 cm or 20 × 10 cm may also be used instead of microscope slides.

15.2.1.2 Developing tank — a tall form beaker of at least 10 cm height.

15.2.1.3 Visualization tank — A dry beaker or tank saturated with iodine vapour by placing a few crystals at the bottom and leaving for an hour.

15.2.2 Apparatus

15.2.2.1 Silica gel containing binder — passing 75-micron IS Sieve; this is a mixture of silica gel (85 g) and calcium sulphate (15 g) as a binder. The mixed material is available commercially.

15.2.2.2 Developing solvent — a mixture of petroleum ether (boiling range 40 to 60°C), diethyl ether and acetic acid in the proportion of 60 : 40 : 2 (v/v).

15.2.3 Procedure — Hold the two microscope slides together face to face and dip them in a slurry of silica gel (45 g) in chloroform-methanol mixture (80 ml + 20 ml). Withdraw the slides, separate them and allow to dry in air for 10 minutes. At one end of the coated slides, apply 5 µl (50 µg) of the oil sample as a one-percent solution in chloroform using a capillary (melting point) tube. Dry briefly, and place the slide in the beaker containing 3 ml of the developing solvent mixture. Cover with a watch glass and allow the solvent to travel up to within 1 cm of the top of the plate (about 6 minutes). Remove from the tank, dry in air and place in the visualization tank containing iodine vapour. Occurrence of a spot with an R_f value of about 0.25 shows the presence of castor oil. All other spots

will be above this. A prepared sample of an oil containing one percent of added castor oil may be run alongside for convenience in identifying the spot. The spot shall be noted in the visualization tank, since it fades on removing.

16. TEST FOR THE PRESENCE OF NEEM OIL

16.1 Outline of the Method—A copper strip or foil immersed in *NEEM* oil or any other oil, free of sulphur compounds, admixed with *NEEM* oil and heated to 200°C for 15 to 60 minutes is deposited with bright colours of different nature (reddish brown, magenta, turquoise, peacock green, bright blue, grey and black). More than one colour may be visible on a strip or foil. Any of these colours present indicates the presence of *NEEM* oil.

16.2 Apparatus

16.2.1 Liquid Paraffin Bath

16.2.2 Forceps

16.2.3 Thermometer — 0 to 250°C or 0 to 360°C.

16.2.4 Test Tubes — 25 mm diameter, 150 mm length.

16.3 Reagents

16.3.1 Copper Strips — 75 × 13 × 0.05 mm, spotless, brightly polished.

16.3.2 n-Hexane

16.3.3 Acetone

16.4 Procedure

16.4.1 Take 30 ml of the sample in a test tube. Keep it in a liquid paraffin bath seated on a tripod. Slip a copper strip into the oil contained in the test tube. Heat the bath gradually to 200°C. Continue heating thereafter for at least 15 minutes, maintaining the temperature not exceeding 200°C. Remove the copper foil by forceps, cool, rinse in hexane and acetone contained in beakers successively to remove oily film and dry.

16.4.2 Observe the surface of the strip. Development of any of the colours (see 16.1) indicates the presence of *NEEM* oil.

16.4.3 Perform a blank test under similar conditions with both pure *NEEM* oil and pure oil in which admixture with *NEEM* oil is being tested. In the absence of *NEEM* oil, the copper strip shall retain its original colour. In pure *NEEM* oil or other oil with 9 to 10 percent of *NEEM* oil admixture, copper strip shall be covered by almost black film or crust. At lower levels, other colours (see 16.1) shall appear.

16.5 Sensitivity — This test is sensitive to the extent of 0.5 percent of *NEEM* oil in other oils.

16.6 Applicability — The method is applicable to both *NEEM* seed oil and *NEEM* kernel oil.

17. TEST FOR THE PRESENCE OF OTHER OILS IN CASTOR OIL

17.0 General — This method, using thin layer chromatography (TLC) technique, is applicable to castor oil contaminated with other oils like groundnut oil, mustard oil, sesame oil, tallow, etc.

17.1 Outline of the Method — Castor oil sample under test is applied to silica gel coated TLC plate and eluted with a solvent system. Pure castor oil gives two spot whereas castor oil adulterated with any other oil gives three or more prominent spots.

17.2 Reagents

17.2.1 Silica Gel G Plates — 20 × 20 cm having 250 nm thick layers.

17.2.2 Silver Nitrate Solution — 5 percent solution in 50 percent aqueous alcohol.

17.2.3 Chloroform

17.2.4 Solvent System — benzene: 95 percent ethanol: acetic acid:: 98.8:1.6:0.5.

17.2.5 Alcoholic Ortho-phosphoric Acid Solution — 50 percent (v/v).

17.2.6 Reference Oil — Sample of uncontaminated and undamaged castor seeds shall be extracted with *n*-hexane and the extract obtained evaporated under vacuum until the residual oil is completely free from solvent.

17.3 Apparatus

17.3.1 TLC Equipment of the Stahl Design or Equivalent.

17.3.2 Drying Oven

17.3.3 Desiccator

17.3.4 TLC Pipettes — 50 µl capacity.

17.4 Procedure

17.4.1 Preparation of the TLC Plate — Prepare a slurry of 30 g of silica gel G in 60 ml of water and spread evenly on five 20 × 20 cm glass plates using TLC equipment adjusted to obtain a 250 nm thick coating. Dry the silica gel G plates for 1 h at 110°C and cool to room temperature. Spray uniformly the silver nitrate solution until the plates appear wet. Dry the plates again at 110°C for 15 minutes. Cool to room temperature and store the plates over silica gel in a desiccator.

17.4.2 Preparation of the Test Solution — Prepare the following solutions in chloroform :

- a) One percent solution of the oil to be tested,
- b) One percent solution of the reference oil, and
- c) One percent solution of mixture (the reference oil containing 3 percent of the other oil).

17.4.3 TLC Procedure

17.4.3.1 Mark the starting point on the prepared TLC plate.

17.4.3.2 Apply a known volume of up to 50 µl of the sample solution to one starting point on the TLC plate.

17.4.3.3 Apply the same volume of the test solution containing the reference oil to one starting point on the TLC plate.

17.4.3.4 Apply the same volume of the test solution containing the reference oil/other oil mixture to one starting point on the plate.

17.4.4 Elution of Oils

17.4.4.1 Allow the solvent to evaporate. Place the TLC plate in a developing vessel containing solvent system benzene : ethanol (95 percent v/v) : acetic acid :: 98·8 : 1·6 : 0·5. Running time is about 35 to 40 minutes to run 18 cm.

17.4.4.2 Dry the plates in an oven at 110°C for 5 minutes.

17.4.4.3 Spray the plates with the alcoholic orthophosphoric acid solution and char the plates in the oven at 150°C for 15 minutes.

17.4.4.4 The oil spots would then appear. Pure castor oil gives two brown spots. A sample adulterated with 3 percent groundnut oil would give two brown and one grey prominent spots. Castor oil adulterated with other oils gives three or more prominent spots.

17.5 Applicability — Peroxides and free fatty acids do not interfere in the test.

17.6 Sensitivity — This test is sensitive to detect the presence of other oils in castor oil to the extent of 3 percent.

18. TEST FOR THE PRESENCE OF ANIMAL FAT IN VEGETABLE OILS (PHYTOSTEROL ACETATE MELTING POINT TEST)

18.0 General — The quantity of the acetate of sterols obtained is very small. Absolute purity of the chemicals used and cleanliness of the apparatus are needed to ensure successful crystallization of the acetate.

18.0.1 The sterols present in oil or fat are first converted into digitonides by treatment with an alcoholic solution of digitonin. The digitonides are then converted into acetates by boiling with acetic anhydride. The acetate of the sterols is purified by repeated crystallization in alcohol and melting point determined.

18.1 Apparatus

18.1.1 Water-Jacketed Filtering Apparatus — consisting of a 100-ml fine grade sintered glass filter passing through a rubber cork fitted in the collared bung hole of an enamelled water-bath which serves as a hot water jacket around the filter (*see Note*). The stem of the filter also passes through a cork which fits the filtering flask, the side tube of the flask being connected by pressure tubing to a vacuum pump (*see Fig. 1*).

NOTE — An enamelled-iron electric lamp shade suitably collared to hold a rubber cork will serve as water-bath.

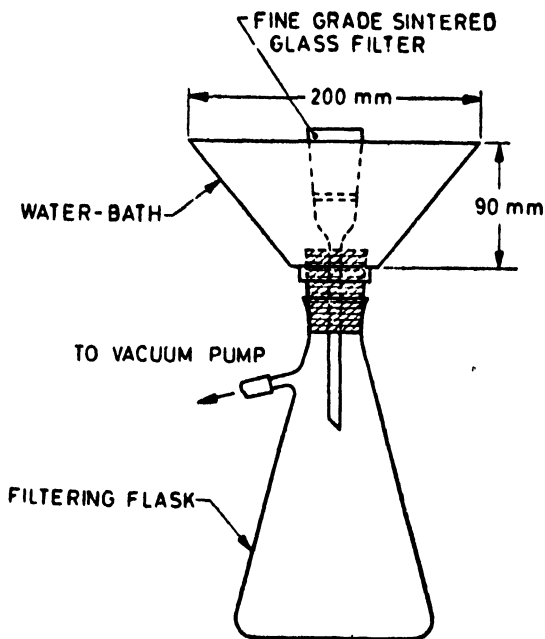


FIG. 1 WATER-JACKETED FILTERING APPARATUS

18.1.2 Witt's Filtering Apparatus — consisting of a medium or a micro sintered glass filter assembled as shown and connected to a suitable vacuum pump; intended for carrying out filtration of the acetate of sterols after crystallization (see Fig. 2).

NOTE — All the filters should be washed with chromic acid, thoroughly flushed with water, followed by alcohol and ether before starting each experiment.

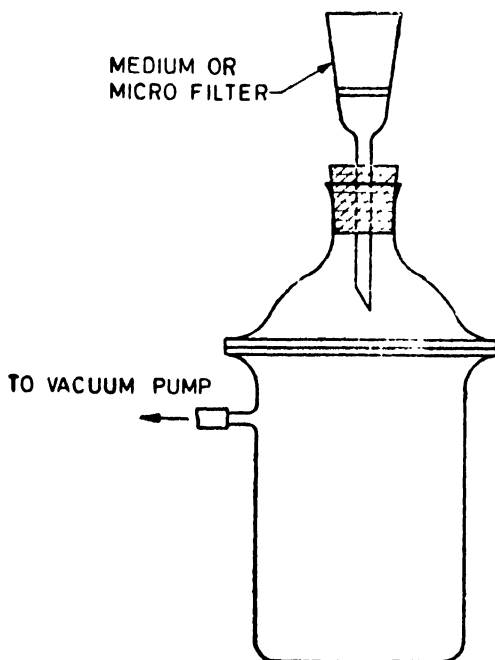


FIG. 2 WITT'S FILTERING APPARATUS

18.1.3 Melting Point Apparatus (Junge's) — consisting of a capillary tube containing a compact column of the acetate sterols attached to a standard thermometer (100 to 150°C graduated to 0.2°C) by a rubber band in such a way that the substance inside the capillary is as close to the bulb of the thermometer as possible. The thermometer is immersed in glycerine so as to cover the effective mercury column in the inner co-axial tube of the apparatus. The outer tube which surrounds the inner tube is filled with sulphuric acid and is provided with a side arm so that when the apparatus is heated at the lowest point by a micro burner the acid circulates through the main body and the side tube. Lagging the tube, in which the sulphuric acid descends, with asbestos cord facilitates even heating (see Fig. 3).

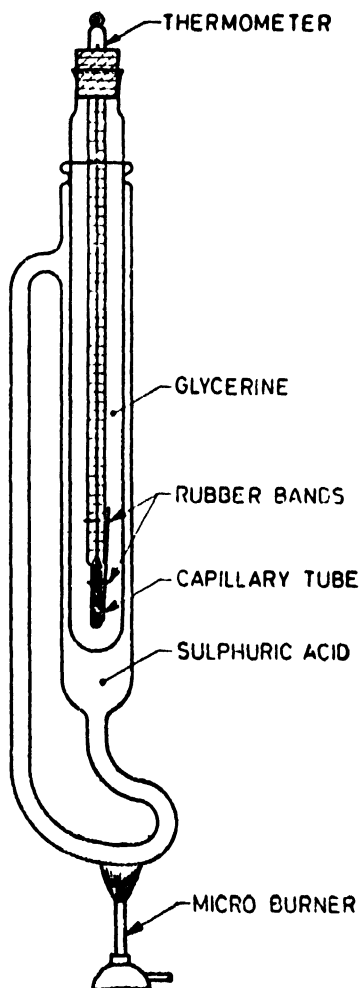


FIG. 3 MELTING POINT APPARATUS (JUNGE'S)

18.1.3.1 An electrically heated melting point apparatus may also be used.

18.1.4 *Vacuum Flask, Wide-Mouthed*

18.1.5 *Vacuum Desiccator*

18.1.6 *Stoke's Tubes of Size 200 × 25 mm*

18.2 Reagents

18.2.1 Digitonin Solution — 1 percent solution in 95 percent ethyl alcohol.

18.2.2 Chloroform

18.2.3 Ether — See IS : 336-1973*.

18.2.4 Absolute Alcohol — See IS : 321-1964†.

18.2.5 Sulphuric Acid — See IS : 266-1961‡.

18.2.6 Glycerine — See IS : 1796-1960§.

18.3 Procedure

18.3.1 Liquefy the fat and filter it free from impurities, moisture and sediment and take about 25 g of the filtered sample of fat, 10 ml of chloroform and 15 ml of digitonin solution in a 250-ml conical flask and shake, by giving a rotatory motion by hand over a water-bath maintained at 60 to 70°C for about 15 minutes.

18.3.2 Heat the 100-ml sintered glass filter (arranged as shown in Fig. 1) to 60 to 70°C for some time, drawing air through the filter bed with gentle suction to dry it. Disconnect suction and pour into the filter the contents of the conical flask after wiping the outside of the flask. Start the vacuum pump and allow the contents to filter (see Note) keeping the mass well stirred during filtration and maintaining the bath at 60 to 70°C. Use gentle suction. Just before the filtration is over and the precipitate is dry, wash it with about 5 ml of chloroform passed through the reaction flask. Repeat the washing with chloroform five or six times, each addition of chloroform being made just before the previous addition is almost filtered and the precipitate has a chance to dry.

NOTE — The filtrate in the first stage is turbid and gives the impression that some of the digitonide has passed through the filter. However, that is not the case. The turbidity is due to the separation of the fat — chloroform — alcohol phases on cooling.

18.3.3 When washing with chloroform is complete, draw air through the filter by means of the vacuum pump till the precipitate appears dry and then place the filter in the vacuum desiccator (at about 2 to 3 mm pressure) for not less than half an hour. Remove the dried digitonide (which will have assumed a paper like texture) from the filter bed with the help of a mounted needle or a pointed glass rod.

*Specification for ether (second revision).

†Specification for absolute alcohol (revised).

‡Specification for sulphuric acid (revised).

§Specification for crude glycerine and refined glycerine.

18.3.4 Transfer the digitonide to the Stoke's tube. Add 5 ml of acetic anhydride and heat over sand-bath by a low flame till it dissolves completely. If necessary, filter the solution while hot in the 100-ml sintered glass filter, rinsing the tube with 0.5 ml of acetic anhydride. To this solution, add 20 ml of 50 percent alcohol, when a milky white precipitate will appear; mix well and allow to crystallize in ice overnight. Filter off the crystals through a 30-ml filter over Witt's filtering apparatus (see Fig. 2), wash thoroughly five to six times with cooled 50 percent alcohol and dry by drawing air through the filter with the help of vacuum pump for about half an hour.

18.3.5 Dissolve the crystals off the filter with ether and collect the solution in small test tube (75 × 12 mm). Evaporate the ether by gently warming and blowing in air (use a rubber bulb). Dissolve the dry acetate in the minimum quantity (about 2 to 3 ml) of 95 percent alcohol and allow to crystallize by keeping it in ice for about half an hour. Filter off the crystals through a clean 2-ml sintered glass filter and wash with 0.5 ml of cooled 95 percent alcohol and dry by drawing air through the filter with the help of vacuum pump for about 10 minutes.

18.3.6 Repeat the process given in 18.3.5 to effect a second crystallization and dry the filter with the acetate crystals, overnight, in a vacuum desiccator.

18.3.7 After taking the acetate crystals in 3 or 4 melting point capillary tubes, seal the ends of the tubes. Attach one of the capillary tubes to the bulb of the thermometer by means of rubber bands and adjust the melting point apparatus as shown in Fig. 3. Heat the apparatus by a micro-burner slowly so that the temperature rises very gradually and steadily. From 110°C onwards, the heating should be so regulated that the rise in temperature is at a rate of not more than 0.5°C per minute.

18.3.8 The temperature at which complete fusion takes place is taken as the melting point of the sterol acetate. Determine the melting point of two more capillary tubes as described in 18.3.7 for confirmation.

18.4 The melting points of sterol acetates from various oils and fats as also their major sterols are as follows :

<i>Fat/Oil</i>	<i>Major Sterol</i>	<i>Melting Point of Sterol Acetate</i>
Animal fat	Cholesterol	114.6°C
Groundnut oil	β-sitosterol	125 to 127°C
Hydrogenated groundnut oil		
Cottonseed oil		
Coconut oil	Stigmasterol	144°C
Mustard oil	Brassicasterol	158°C

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